

SEQUENCE LISTING

<110> HOSHINO, Tatsuo
 OJIMA, Kazuyuki
 SETOGUCHI, Yutaka

<120> ASTAXANTHIN SYNTHETASE

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<140> US/09/518,386

<141> 2000-03-03

<150> EP 99104668.1

<151> 1999-03-09

<150> EP 00101666.6

<151> 2000-02-01

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<170> PatentIn Ver. 2.1

<210> 1

<211> 557

<212> PRT

<213> Phaffia rhodozyma

<220>

<221> TRANSIT

<222> (1)..(26)

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Gly Asn Phe Leu Asp Ile Leu Ser Ala Arg Thr Gly Glu Glu His Ala
 50 55 60

Lys Tyr Arg Glu Lys Tyr Gly Ser Thr Leu Arg Phe Ala Gly Ile Ala
 65 70 75 80

Gly Ala Pro Val Leu Asn Ser Thr Asp Pro Lys Val Phe Asn His Val
 85 90 95

Met Lys Glu Ala Tyr Asp Tyr Pro Lys Pro Gly Met Ala Ala Arg Val
 100 105 110

Leu Arg Ile Ala Thr Gly Asp Gly Val Val Thr Ala Glu Gly Glu Ala
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His Lys Arg His Arg Arg Ile Met Ile Pro Ser Leu Ser Ala Gln Ala
 130 135 140

Val Lys Ser Met Val Pro Ile Phe Leu Glu Lys Gly Met Glu Leu Val
 145 150 155 160

Asp Lys Met Met Glu Asp Ala Ala Glu Lys Asp Met Ala Val Gly Glu
 165 170 175

Ser Ala Gly Glu Lys Lys Ala Thr Arg Leu Glu Thr Glu Gly Val Asp
 180 185 190

Val Lys Asp Trp Val Gly Arg Ala Thr Leu Asp Val Met Ala Leu Ala
 195 200 205

Gly Phe Asp Tyr Lys Ser Asp Ser Leu Gln Asn Lys Thr Asn Glu Leu
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Tyr Val Ala Phe Val Gly Leu Thr Asp Gly Phe Ala Pro Thr Leu Asp
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Ser Phe Lys Ala Ile Met Trp Asp Phe Val Pro Tyr Phe Arg Thr Met
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Arg Val Gly Ile Glu Leu Met Glu Gln Lys Lys Gln Ala Val Leu Gly

275

280

285

Ser Ala Ser Asp Gln Ala Val Asp Lys Lys Asp Val Gln Gly Arg Asp
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Ile Leu Ser Leu Leu Val Arg Ala Asn Ile Ala Ala Asn Leu Pro Glu
 305 310 315 320

Ser Gln Lys Leu Ser Asp Glu Glu Val Leu Ala Gln Ile Ser Asn Leu
 325 330 335

Leu Phe Ala Gly Tyr Glu Thr Ser Ser Thr Val Leu Thr Trp Met Phe
 340 345 350

His Arg Leu Ser Glu Asp Lys Ala Val Gln Asp Lys Leu Arg Glu Glu
 355 360 365

Ile Cys Gln Ile Asp Thr Asp Met Pro Thr Leu Asp Glu Leu Asn Ala
 370 375 380

Leu Pro Tyr Leu Glu Ala Phe Val Lys Glu Ser Leu Arg Leu Asp Pro
 385 390 395 400

Pro Ser Pro Tyr Ala Asn Arg Glu Cys Leu Lys Asp Glu Asp Phe Ile
 405 410 415

Pro Leu Ala Glu Pro Val Ile Gly Arg Asp Gly Ser Val Ile Asn Glu
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Val Arg Ile Thr Lys Gly Thr Met Val Met Leu Pro Leu Phe Asn Ile
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Asn Arg Ser Lys Phe Ile Tyr Gly Glu Asp Ala Glu Glu Phe Arg Pro
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Glu Arg Trp Leu Glu Asp Val Thr Asp Ser Leu Asn Ser Ile Glu Ala
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Pro Tyr Gly His Gln Ala Ser Phe Ile Ser Gly Pro Arg Ala Cys Phe
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Gly Trp Arg Phe Ala Val Ala Glu Met Lys Ala Phe Leu Phe Val Thr
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Leu Arg Arg Val Gln Phe Glu Pro Ile Ile Ser His Pro Glu Tyr Glu
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 101

Thr Gly Ala Leu Gly Leu Ala Ala Phe Ser Trp Ala Ser Ile Ala Phe
 10 15 20

ttc agt ctt tac ctc gct ccg agg cga tct tca ctg tat aac ctt cag
 149

Phe Ser Leu Tyr Leu Ala Pro Arg Arg Ser Ser Leu Tyr Asn Leu Gln
 25 30 35

gca aac atc gcc gcc aac ctg cct gaa tct caa aag ctg tcc gat gag
1013

Ala Asn Ile Ala Ala Asn Leu Pro Glu Ser Gln Lys Leu Ser Asp Glu
315 320 325

gag gta ctc gct cag atc agt aac ctg tta ttt gct gga tat gaa act
1061

Glu Val Leu Ala Gln Ile Ser Asn Leu Leu Phe Ala Gly Tyr Glu Thr
330 335 340

tct tcg aca gtc ttg aca tgg atg ttt cac cga ctc tca gaa gac aaa
1109

Ser Ser Thr Val Leu Thr Trp Met Phe His Arg Leu Ser Glu Asp Lys
345 350 355

gcc gtt cag gat aaa ctt cga gaa gaa att tgt cag atc gac acg gat
1157

Ala Val Gln Asp Lys Leu Arg Glu Glu Ile Cys Gln Ile Asp Thr Asp
360 365 370 375

atg cct acg cta gac gaa ctt aat gcg ttg cct tat ctc gaa gcg ttt
1205

Met Pro Thr Leu Asp Glu Leu Asn Ala Leu Pro Tyr Leu Glu Ala Phe
380 385 390

gtt aag gag tct ctt cgt cta gac cct cct agt ccg tat gct aac cgt
1253

Val Lys Glu Ser Leu Arg Leu Asp Pro Pro Ser Pro Tyr Ala Asn Arg
395 400 405

gaa tgc tta aag gat gaa gac ttc atc cca ctt gcc gag cct gtc att
1301

Glu Cys Leu Lys Asp Glu Asp Phe Ile Pro Leu Ala Glu Pro Val Ile
410 415 420

ggc cga gat ggg tcg gtc atc aac gag gtc cgg atc acg aaa gga acg
1349

Gly Arg Asp Gly Ser Val Ile Asn Glu Val Arg Ile Thr Lys Gly Thr
425 430 435

atg gtc atg ctt ccg ttg ttc aac atc aat cgt tca aag ttc att tat
1397

Met Val Met Leu Pro Leu Phe Asn Ile Asn Arg Ser Lys Phe Ile Tyr

455

Gly Glu Asp Ala Glu Glu Phe Arg Pro Glu Arg Trp Leu Glu Asp Val
460 465 470

Thr Asp Ser Leu Asn Ser Ile Glu Ala Pro Tyr Gly His Gln Ala Ser
475 480 485

Phe Ile Ser Gly Pro Arg Ala Cys Phe Gly Trp Arg Phe Ala Val Ala
490 495 500

Glu Met Lys Ala Phe Leu Phe Val Thr Leu Arg Arg Val Gln Phe Glu
505 510 515

Pro Ile Ile Ser His Pro Glu Tyr Glu His Ile Thr Leu Ile Ile Ser
520 525 530 535

Arg Pro Arg Ile Val Gly Arg Glu Lys Glu Gly Tyr Gln Met Arg Leu
540 545 550

Gln Val Lys Pro Val Glu
555

gggcaggcgc tatgacttct acgtcgtcta tcgtcgtctt ggactctctt cttaccctat
1856

atattattcc atccgaaaaa aaaaaaaaaa aaaaaaaaaa aaaaagcggc cgctcgagcc
1916

ggctcgtgcc gaattc
1932

<210> 3
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<212> PRT
<213> Phaffia rhodozyma

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35 40 45
Gly Asn Phe Leu Asp Ile Leu Ser Ala Arg Thr Gly Glu Glu His Ala
50 55 60
Lys Tyr Arg Glu Lys Tyr Gly Ser Thr Leu Arg Phe Ala Gly Ile Ala
65 70 75 80
Gly Ala Pro Val Leu Asn Ser Thr Asp Pro Lys Val Phe Asn His Val
85 90 95
Met Lys Glu Ala Tyr Asp Tyr Pro Lys Pro Gly Met Ala Ala Arg Val
100 105 110
Leu Arg Ile Ala Thr Gly Asp Gly Val Val Thr Ala Glu Gly Glu Ala
115 120 125
His Lys Arg His Arg Arg Ile Met Ile Pro Ser Leu Ser Ala Gln Ala
130 135 140
Val Lys Ser Met Val Pro Ile Phe Leu Glu Lys Gly Met Glu Leu Val
145 150 155 160
Asp Lys Met Met Glu Asp Ala Ala Glu Lys Asp Met Ala Val Gly Glu
165 170 175
Ser Ala Gly Glu Lys Lys Ala Thr Arg Leu Glu Thr Glu Gly Val Asp
180 185 190
Val Lys Asp Trp Val Gly Arg Ala Thr Leu Asp Val Met Ala Leu Ala
195 200 205
Gly Phe Asp Tyr Lys Ser Asp Ser Leu Gln Asn Lys Thr Asn Glu Leu
210 215 220
Tyr Val Ala Phe Val Gly Leu Thr Asp Gly Phe Ala Pro Thr Leu Asp
225 230 235 240

Ser	Phe	Lys	Ala	Ile	Met	Trp	Asp	Phe	Val	Pro	Tyr	Phe	Arg	Thr	Met	245	250	255
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Arg	Val	Gly	Ile	Glu	Leu	Met	Glu	Gln	Lys	Lys	Gln	Ala	Val	Leu	Gly	275	280	285
Ser	Ala	Ser	Asp	Gln	Ala	Val	Asp	Lys	Lys	Asp	Val	Gln	Gly	Arg	Asp	290	295	300
Ile	Leu	Ser	Leu	Leu	Val	Arg	Ala	Asn	Ile	Ala	Ala	Asn	Leu	Pro	Glu	305	310	315
Ser	Gln	Lys	Leu	Ser	Asp	Glu	Glu	Val	Leu	Ala	Gln	Ile	Ser	Asn	Leu	325	330	335
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His	Arg	Leu	Ser	Glu	Asp	Lys	Ala	Val	Gln	Asp	Lys	Leu	Arg	Glu	Glu	355	360	365
Ile	Cys	Gln	Ile	Asp	Thr	Asp	Met	Pro	Thr	Leu	Asp	Glu	Leu	Asn	Ala	370	375	380
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Pro	Tyr	Gly	His	Gln	Ala	Ser	Phe	Ile	Ser	Gly	Pro	Arg	Ala	Cys	Phe	485	490	495
Gly	Trp	Arg	Phe	Ala	Val	Ala	Glu	Met	Lys	Ala	Phe	Leu	Phe	Val	Thr	500	505	510
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His	Ile	Thr	Leu	Ile	Ile	Ser	Arg	Pro	Arg	Ile	Val	Gly	Arg	Glu	Lys	530	535	540
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3969

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for expression of the AST gene in E. coli

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<220>
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primer for expression of the AST gene in E. coli

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47

<210> 7
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
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primer for expression of a modified AST gene in E.
coli

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<210> 8
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<220>
<223> Description of Artificial Sequence: 5' antisense
primer for expression of a modified AST gene in E.
coli

<400> 8
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<210> 9
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
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primer for expression of a modified AST gene in E.
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<210> 10
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<212> DNA
<213> Artificial Sequence

<220>
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primer for cloning of the AST gene

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<210> 11
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<210> 12
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<220>
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<210> 13
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<220>
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primer for cloning the genomic AST gene

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<210> 14
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<210> 15
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<220>
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primer for RT-PCR of the AST gene

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<210> 16
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sense primer for cloning of the TPI gene

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10066007.000100

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<210> 17
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<212> DNA
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<220>
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<220>
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primer for cloning of the TPI terminator

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<210> 20
<211> 27
<212> DNA
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<220>
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walking primer for cloning of the TPI promoter

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<210> 21
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Nested walking
primer for cloning of the TPI promoter

<400> 21
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<210> 22

<211> 28
<212> DNA
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<220>
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for construction of the TPI promoter cassette

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28

<210> 23
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<220>
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primer for construction of the TPI promoter
cassette

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34

<210> 24
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<212> DNA
<213> Artificial Sequence

<220>
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for construction of the TPI terminator cassette

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<210> 25

10066007.030103

<211> 28
<212> DNA
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28

<210> 26
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<221> misc_feature
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10065007.020102

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for construction of a partial AMY cassette

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<210> 29

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primer for construction of a partial AMY cassette

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<210> 30

<211> 28

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Sense primer
for construction of the AST cassette

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28

<210> 31

<211> 28

<212> DNA

<213> Artificial Sequence

Figure 1 consists of 12 line graphs, labeled (a) through (l), arranged in a single column. Each graph plots a different physiological or behavioral parameter over a 10-minute period. The y-axis for all graphs ranges from 0 to 100. The x-axis for all graphs ranges from 0 to 10 minutes. The graphs show a general decrease in most parameters during the intervention period, with some parameters showing a slight increase or stabilization.

- (a) HR (b/min): Shows a decrease from approximately 75 to 65 b/min.
- (b) BP (mmHg): Shows a decrease from approximately 120 to 100 mmHg.
- (c) SV (ml): Shows a decrease from approximately 70 to 60 ml.
- (d) SVI (ml/m²): Shows a decrease from approximately 70 to 60 ml/m².
- (e) CO (l/min): Shows a decrease from approximately 5.5 to 4.5 l/min.
- (f) COI (l/min/m²): Shows a decrease from approximately 4.5 to 3.5 l/min/m².
- (g) TPR (mmHg/l/min): Shows a decrease from approximately 18 to 15 mmHg/l/min.
- (h) TPR (mmHg/l/min): Shows a decrease from approximately 18 to 15 mmHg/l/min.
- (i) TPR (mmHg/l/min): Shows a decrease from approximately 18 to 15 mmHg/l/min.
- (j) TPR (mmHg/l/min): Shows a decrease from approximately 18 to 15 mmHg/l/min.
- (k) TPR (mmHg/l/min): Shows a decrease from approximately 18 to 15 mmHg/l/min.
- (l) TPR (mmHg/l/min): Shows a decrease from approximately 18 to 15 mmHg/l/min.

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28
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<220>
<223> Description of Artificial Sequence: Sense primer
for confirmation of integration at the AMY locus
by PCR analysis

```
<400> 32
ctctcctgtt cacaaaaaca
20
```